ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit

Protocol

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1 Introduction

Chapter Summary

In This Chapter The following topics are covered in this chapter:

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About the Kit

Reagent Requirements

- The ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS, requires **new** instrument (matrix) files for the ABI PRISM® 310 Genetic Analyzer, ABI PRISM® 377 DNA Sequencers, and ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel installed and **new** spectral calibrations for the ABI PRISM® 3700 DNA Analyzer and the ABI PRISM® 3100 Genetic Analyzer:
  - The 310, 377, and 373 instruments require the ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421) for instrument (matrix) file generation.
  - The 3700 instrument requires ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309) for spectral calibration.
  - The 3100 instrument requires ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303) for spectral calibration.
- The dRhodamine Matrix Standards and Matrix Standard Set DS-01 are not compatible with BigDye™ terminators v3.0, dGTP BigDye™ terminators v3.0, or BigDye™ primers v3.0.
- The alcohol precipitation methods are different from those recommended for the original BigDye terminators.
- There are new mobility files for all existing platforms.
- The basecallers are the same.

BigDye Terminator v3.0 Ready Reaction Kit

The ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit provides AmpliTaq® DNA Polymerase, FS, BigDye™ terminators v3.0, and all the required components for the sequencing reaction.

In the Ready Reaction format, the dye terminators, deoxynucleoside triphosphates, AmpliTaq DNA Polymerase, FS, magnesium chloride, and buffer are premixed into a single tube of Ready Reaction Mix and are ready to use. These reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, on polymerase chain reaction (PCR) fragments, and on large templates, *e.g.*, BAC clones.

The dNTP mix includes dITP in place of dGTP to minimize band compressions. The dNTP mix also uses dUTP in place of dTTP. dUTP improves the incorporation of the T terminator and results in a better T pattern.

Cycle Sequencing with AmpliTaq DNA Polymerase, FS

The kit formulation contains the sequencing enzyme AmpliTaq DNA Polymerase, FS. This enzyme is a variant of *Thermus aquaticus* DNA polymerase that contains a point mutation in the active site. This results in less discrimination against dideoxynucleotides.

This enzyme also has a second mutation in the amino terminal domain that virtually eliminates the 5´→3´ nuclease activity of AmpliTaq DNA Polymerase. The enzyme has been formulated with a thermally stable inorganic pyrophosphatase to eliminate problems associated with pyrophosphorolysis.

Cycle sequencing protocols that rely on the use of AmpliTaq DNA Polymerase, FS offer the following advantages over traditional sequencing methods:

- Less hands-on operation
- No alkaline denaturation step required for double-stranded DNA
- Same protocol for both single- and double-stranded templates
- Less starting template needed
- More reproducible results

BigDye Terminator v3.0 Appearance on the 377 or 373 Instrument Gel Image

The dye/base relationships and colors of the BigDye terminators v3.0 as they appear on the gel image are shown below for the 377 and 373 instruments.

Base	Terminator	Color of Bands on ABI PRISM 377 or 373 Instrument Gel Image
A	V3 Dye 2	Green
C	V3 Dye 4	Red
G	V3 Dye 1	Blue
T	V3 Dye 3	Yellow

Comparing BigDye Chemistries

Data generated with BigDye terminators v3.0 gives more even peak-height patterns than data generated with BigDye terminators (original) and BigDye terminators v2.0.

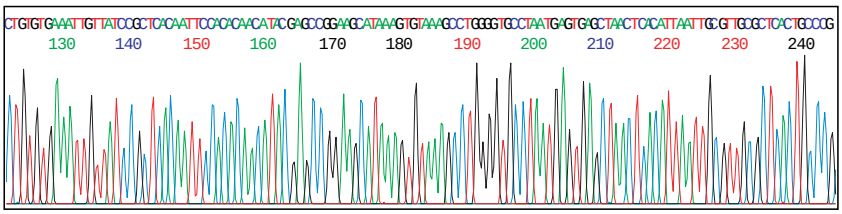

Figure 1-1 Region of pGEM®-3Zf(+) sequenced with BigDye terminators (original)

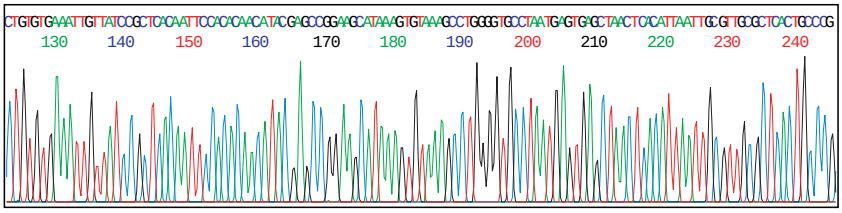

Figure 1-2 Region of pGEM-3Zf(+) sequenced with BigDye terminators v2.0

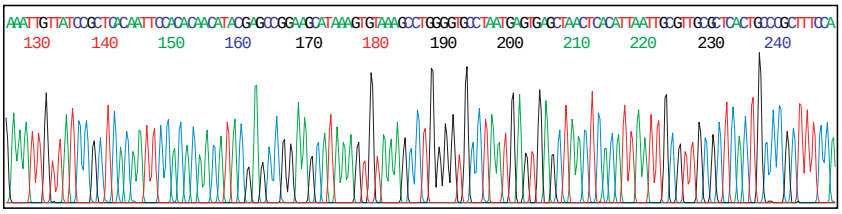

Figure 1-3 Region of pGEM-3Zf(+) sequenced with BigDye terminators v3.0

Instruments

Instrument Platforms

The ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit is for use with the following instruments:

- ABI PRISM® 3700 DNA Analyzer
- ABI PRISM® 3100 Genetic Analyzer
- ABI PRISM® 310 Genetic Analyzer
- ABI PRISM® 377 DNA Sequencer (all models[1])

This kit can also be used with ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel installed.[2] Refer to the *ABI PRISM BigDye Filter Wheel User Bulletin* (P/N 4304367) for more information.

General instructions are given for using the kit reagents to generate samples for these instruments. For more detailed instructions, refer to the appropriate instrument user's manual or chemistry guide.

IMPORTANT This kit is not designed for use with ABI PRISM® 373 DNA Sequencers and ABI PRISM® 373 DNA Sequencers with XL Upgrade that do not have the ABI PRISM BigDye Filter Wheel.

Thermal Cyclers

The protocols provided in this document were optimized using Applied Biosystems thermal cyclers, including:

- GeneAmp® PCR Systems 9700, 9600, and 2400
- DNA Thermal Cycler 480
- DNA Thermal Cycler (TC1)

If you use a thermal cycler not manufactured by Applied Biosystems, you may need to optimize thermal cycling conditions. Ramping time is very important. If the thermal ramping time is too fast (>1°/second), poor (noisy) data may result.

1. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.
2. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Required Software

Dye/Filter Sets and Matrix Standards for the 310, 377, and 373 Instruments

The dye/filter sets and matrix standards required for the 310, 377, and 373 instruments are listed in the table below.

IMPORTANT The instrument (matrix) file for the BigDye terminators v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Instrument	Dye/Filter Set	Standards for Instrument (Matrix) File Generation
310 Genetic Analyzer	Filter Set E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)
377 DNA Sequencers[a]	Filter Set E	
373 DNA Sequencers with the BigDye Filter Wheel[b]	Filter Set A	

a. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

b. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Dye Sets and Spectral Standards for the 3700 and 3100 Instruments

The dye sets and spectral standards required for the 3700 and 3100 instruments are listed in the table below.

IMPORTANT Spectral calibrations for the BigDye terminators v3.0 are not compatible with the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Instrument	Dye Set	Standards for Spectral Calibration
3700 DNA Analyzer	D	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309)
3100 Genetic Analyzer	Z	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303)

Instructions

For Generating Matrices

- For the 377 and 310 instruments, refer to the product insert for instructions on using the ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421) to generate matrices.
- For the 373 instruments, contact Technical Support for instructions on using the ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421) to generate matrices.

For Performing Spectral Calibrations

- For the 3700 instrument, refer to the product insert for instructions on using the ABI PRISM 3700 BigDye Terminator v3.0 Sequencing Standard (P/N 4390309) to perform spectral calibration.
- For the 3100 instrument, refer to the product insert for instructions on using the ABI PRISM BigDye Terminator v3.0 Sequencing Standard (P/N 4390303) to perform spectral calibration.

Dye Set/Primer (Mobility) Files

Available in Two Places

To analyze sequencing data generated with BigDye chemistries v3.0, you need dye set/primer (mobility) files that were created for v3.0 chemistries. The dye set/primer (mobility) files can be obtained from two places:

- The files can be installed from the two CD-ROMs or one floppy disk enclosed in the v3.0 matrix and sequencing standards listed below. See page 1-8 for instructions.
  - ABI PRISM BigDye Matrix Standards v3.0 (P/N 4390421)
  - ABI PRISM BigDye Terminator v3.0 Sequencing Standard (P/N 4390303)
  - ABI PRISM 3700 BigDye Terminator v3.0 Sequencing Standard (P/N 4390309)
- The files can be downloaded from the Internet. See page 1-8 for instructions.

Installing Files from the CD-ROMs or Floppy Disk Enclosed in the v3.0 Matrix and Sequencing Standards

If you wish to analyze your data using a ...	and your data was collected on a...	Refer to the CD-ROM or floppy disk labeled...
computer with the Windows NT® platform	3700, 3100, 310, or 377 instrument	PN 4326478, For Windows NT platform
Macintosh® computer with a CD-ROM drive	310, 377, 373 instrument	PN 4326479, For Macintosh platform
Macintosh computer with a floppy drive	310, 377, 373 instrument	PN 4326480, For Macintosh platform

Downloading Files from the Internet

Dye set/primer (mobility) files can be downloaded from our website:

http://www.appliedbiosystems.com/techsupp/swpps/SAsw.html

If you do not have access to the Internet, you can get the files from Applied Biosystems Technical Support, or from your local field applications specialist (call your local sales office for more information).

Reagents and Storage

Available Kits

The following kits are available:

Kit	Number of Reactions	Part Number
The ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS	24	4390236
	100	4390242
	1000	4390244
	5000	4390246
	25000	4390253

Description of Reagents

A description of the kit reagents is listed below.

- Terminator Ready Reaction Mix:
  - A-BigDye Terminator v3.0
  - C-BigDye Terminator v3.0
  - G-BigDye Terminator v3.0
  - T-BigDye Terminator v3.0
  - Deoxynucleoside triphosphates (dATP, dCTP, dITP, dUTP)
  - AmpliTaq DNA Polymerase, FS
  - $MgCl_2$
  - Tris-HCl buffer, pH 9.0
- pGEM®-3Zf(+) double-stranded DNA Control Template, 0.2 µg/µL
- –21 M13 Control Primer (forward), 0.8 pmol/µL

Storage and Use of the Kit

- Store the kit at –15 to –25 °C.
- Avoid excess (*i.e.,* no more than 5–10) freeze-thaw cycles. Aliquot reagents in smaller amounts if necessary.
- Before each use of the kit, allow the frozen stocks to thaw at room temperature (do not heat).

IMPORTANT Mix each stock thoroughly and then centrifuge briefly to collect all the liquid at the bottom of each tube.

- Whenever possible, thawed materials should be kept on ice during use. Do not leave reagents at room temperature for extended periods.

Materials Supplied by the User

Overview In addition to the reagents supplied in this kit, other items are required.

This section lists general materials needed for:

- Cycle sequencing
- Purifying extension products

Note Many of the items listed in this section are available from major laboratory suppliers (MLS) unless otherwise noted. Equivalent sources may be acceptable where noted.

Refer to the individual instrument protocols for the specific items needed for each instrument.

⚠ WARNING CHEMICAL HAZARD. Before handling the chemical reagents needed for cycle sequencing, read the safety warnings on the reagent bottles and in the manufacturers' Material Safety Data Sheets (MSDSs). Always wear protective equipment (lab coat, safety glasses, and chemical-resistant gloves) when handling chemicals. Dispose of waste in accordance with all local, state/provincial, and national environmental and health regulations.

Materials for Cycle Sequencing

The table below lists the plates or tubes required for the recommended Applied Biosystems thermal cyclers (page 1-5).

Thermal Cycler	Plate or Tube	Applied Biosystems Part Number
GeneAmp® PCR System 9700	MicroAmp® 96-Well Reaction Plate	N801-0560
	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 or N801-0535
	ABI PRISM™ Optical Adhesive Cover Starter Pack or ABI PRISM® Optical Adhesive Covers	4313663 or 4311971
GeneAmp® PCR System 9600	MicroAmp® 96-Well Reaction Plate	N801-0560
	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 N801-0535
	ABI PRISM™ Optical Adhesive Cover Starter Pack or ABI PRISM® Optical Adhesive Covers	4313663 or 4311971
GeneAmp® PCR System 2400	MicroAmp® Reaction Tubes, 0.2-mL	N801-0533
	MicroAmp® Caps, 12 or 8/strip	N801-0534 N801-0535
DNA Thermal Cycler 480[a]	GeneAmp® Thin-Walled Reaction Tubes, 0.5-mL	N801-0537
	GeneAmp® Thin-Walled Reaction Tubes with Flat Cap	N801-0737
DNA Thermal Cycler (TC1)[a]	GeneAmp® Thin-Walled Reaction Tubes, 0.5-mL	N801-0537

a. The DNA Thermal Cycler (TC1) and the DNA Thermal Cycler 480 thermal cyclers require mineral oil that can be obtained from Applied Biosystems (P/N 0186-2302)

Materials for Purifying Extension Products

Method	Material	Supplier
Ethanol/Sodium Acetate Precipitation **Note** For 96-well reaction plates and microcentrifuge tubes.	Ethanol (EtOH), non-denatured, 95% Sodium acetate (NaOAc), 3 M, pH 4.6 Aluminum foil tape, adhesive-backed	MLS Applied Biosystems (P/N 400320) 3M (Scotch Tape P/N 431 or 439)[a]
Ethanol Precipitation **Note** For 96-well reaction plates and microcentrifuge tubes.	Ethanol (EtOH), non-denatured, 95% Aluminum foil tape, adhesive-backed	MLS 3M (Scotch Tape P/N 431 or 439)[a]
Ethanol/EDTA Precipitation **Note** For 384-well reaction plates.	Ethanol (EtOH), non-denatured, 95% EDTA, 250 mM Aluminum foil tape, adhesive-backed	MLS MLS 3M (Scotch Tape P/N 431 or 439)[a]
Ethanol/EDTA/ Sodium Acetate Precipitation **Note** For 384-well reaction plates	Ethanol (EtOH), non-denatured, 95% Sodium acetate (NaOAc), 3 M, pH 4.6 EDTA, 250 mM Aluminum foil tape, adhesive-backed	MLS Applied Biosystems (P/N 400320) MLS 3M (Scotch Tape P/N 431 or 439)[a]
Plate Column Purification **Note** For 96-well reaction plates	96-Well columns for purification Aluminum foil tape, adhesive-backed	See Chapter 4. 3M (Scotch Tape P/N 431 or 439)[a]
Spin Column Purification	Centri-Sep™ spin column, 1-mL, 32 columns, 100 columns Aluminum foil tape, adhesive-backed	Applied Biosystems P/N 401763, P/N 401762 3M (Scotch Tape P/N 431 or 439)[a]

a. Contact 3M in the USA at (800) 364-3577 for your local 3M representative. Use of other tapes may result in leakage or contamination of the sample.

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠ WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING **CHEMICAL WASTE HAZARD**. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with and inhalation of chemical waste. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing).
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current material safety data sheet (MSDS) before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING **CHEMICAL HAZARD**. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
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For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Preparing the Templates 2

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Topic	See Page
Control DNA Templates	2-2
Template Preparation Methods	2-3
Single- and Double-Stranded Templates	2-3
BAC DNA Templates	2-3
PCR Templates	2-3
Use of the Primer Island Transposition Kit	2-5
DNA Quantity	2-6

Control DNA Templates

Using Control DNA

Include a control DNA template as one of the templates in a set of sequencing reactions. The results from the control can help determine whether failed reactions are the result of poor template quality or sequencing reaction failure.

Control DNA Sequence

We recommend M13mp18 as a single-stranded control and pGEM®-3Zf(+) as a double-stranded control. All Applied Biosystems DNA sequencing kits provide pGEM® control DNA. All dye terminator cycle sequencing kits include a –21 M13 forward primer for use in performing all reactions.

The partial sequence of pGEM-3Zf(+) from the –21 M13 forward primer, followed by the ensuing 1000 bases is shown in Appendix B, “Control DNA Sequence.”

An Additional Control Sold Separately

The BigDye™ terminator v3.0 sequencing standard provides an additional control to help in troubleshooting electrophoresis runs. It contains lyophilized sequencing reactions that require only resuspension and denaturation before use.

There are two existing forms of the v3.0 sequencing standard, as shown in the table below. Please use the correct sequencing standard for your instrument. Refer to the product inserts for instructions on using each sequencing standard.

Instrument	Kit	PN
ABI PRISM® 3700 DNA Analyzer	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard	4390309
ABI PRISM® 3100 Genetic Analyzer	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard	4390303
ABI PRISM® 310 Genetic Analyzer		
ABI PRISM® 377 DNA Sequencers[a]		
ABI PRISM® 373 DNA Sequencers with the BigDye™ Filter Wheel[b]		

a. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

b. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Template Preparation Methods

Single- and Double-Stranded Templates

Refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for information on preparing single- and double-stranded templates.

BAC DNA Templates

With larger DNA targets such as bacterial artificial chromosomes (BACs), the quality of DNA template is important to the success of the sequencing reaction. Two methods have given good sequencing results:

- Alkaline lysis[1], with extra phenol extraction and isopropanol precipitation if very clean DNA is desired
- Cesium chloride (CsCl) banding

Commercial Kits

Commercial kits are also available for BAC DNA preparation:

- QIAGEN-tip 100 (QIAGEN: P/N 10043, 25 reactions; 10045, 100 reactions)
- QIAGEN-tip 500 (QIAGEN: P/N 10063, 25 reactions; 10065, 100 reactions)

PCR Templates

Cycle sequencing provides the most reproducible results for sequencing PCR templates. Although PCR fragments can be difficult to denature with traditional sequencing methods, cycle sequencing provides several chances to denature and extend the template, which ensures adequate signal in the sequencing reaction.

Importance of Purifying Product

For optimum results, purify the PCR product before sequencing. In general, any method that removes dNTPs and primers should work. We recommend Centricon®-100 columns (P/N N930-2119). The protocol for using these columns is provided in “Purifying PCR Fragments.”

Refer to the *Automated DNA Sequencing Chemistry Guide* (P/N 4305080) for information on sequencing PCR templates.

1. Marra, M., Weinstock, L.A., and Mardis, E.R. 1996. End sequence determination from large insert cloning using energy transfer fluorescent primers. *Genomic Methods* 6: 1118–1122.

Purifying PCR Fragments

To purify PCR fragments by ultrafiltration:

Step	Action
1	Assemble the Centricon-100 column according to the manufacturer's recommendations.
2	Load 2 mL deionized water onto the column.
3	Add the entire sample to the column.
4	Spin the column at 3000 × *g* in a fixed-angle centrifuge for 10 minutes. **Note** The manufacturer recommends a maximum speed of 1000 × *g*, but 3000 × *g* has worked well in Applied Biosystems laboratories. If you are following the manufacturer's guidelines, increase the time to compensate.
5	Remove the waste receptacle and attach the collection vial.
6	Invert the column and spin it at 270 × *g* for 2 minutes to collect the sample. This should yield approximately 40–60 µL of sample.
7	Add deionized water to bring the purified PCR fragments to the original volume.

Use of the Primer Island Transposition Kit

Overview

The BigDye™ terminators v3.0 are also suitable for sequencing plasmid templates generated using the Primer Island® Transposition Kit (P/N 402984). This kit uses transposons to insert primer binding sites into cloned DNA.

About Transposons

Transposons are mobile genetic elements, regions of DNA capable of inserting themselves (or copies of themselves) into the genome. Transposons encode the proteins that facilitate their insertion into the target DNA.

Inserting Artificial Transposons

This property of transposons can be exploited to place unique primer binding sites randomly throughout any large segment of DNA. These primer sites may be used subsequently as templates for PCR and/or sequencing reactions. Transposon insertion is an alternative to subcloning or primer walking when sequencing a large cloned DNA region.[2,3]

The Primer Island Transposition Kit provides reagents for generating artificial transposon insertions into target DNA *in vitro*. The artificial transposon contains the PI(+) and PI(–) priming sites. The Primer Island reagents are combined with a target DNA of choice and used to transform *Escherichia coli*.

Technique

To identify the *E. coli* carrying the transposon, the transformed bacteria are plated on Luria-Bertani (LB) agar plates containing carbenicillin and trimethoprim antibiotics. Each carbenicillin- and trimethoprim-resistant colony has integrated a copy of the transposon into the target DNA.

Follow the *Primer Island Transposition Kit Protocol* (P/N 402920) for transposon insertion and template preparation.

2. Devine, S.E., and Boeke, J.D. 1994. Efficient integration of artificial transposons into plasmid targets *in vitro*: a useful tool for DNA mapping, sequencing, and functional analysis. *Nucleic Acids Res.* 22: 3765–3772.

3. Devine, S.E., Chissoe, S.L., Eby, Y., Wilson, R.K., and Boeke, J.D. 1997. A transposon-based strategy for sequencing repetitive DNA in eukaryotic genomes. *Genome Res.* 7: 551–563.

DNA Quantity

Quantitating DNA

If possible, quantitate the amount of purified DNA by measuring the absorbance at 260 nm or by some other method.

Template Quantity

The table below shows the amount of template to use in a cycle sequencing reaction.

Template	Quantity
PCR product:	
100–200 bp	1–3 ng
200–500 bp	3–10 ng
500–1000 bp	5–20 ng
1000–2000 bp	10–40 ng
>2000 bp	40–100 ng
Single-stranded	50–100 ng
Double-stranded	200–500 ng
Cosmid, BAC	0.5–1.0 µg

Note In general, higher DNA quantities give higher signal intensities.

The template quantities stated above should work with all primers. You may be able to use even less DNA, especially when sequencing with the –21 M13 primer. The amount of PCR product to use in sequencing will also depend on the length and purity of the PCR product.

Template Volume

Cycle-sequencing reactions are made up in a final volume of 20 µL. The volume allows for up to 8 µL for DNA template and 4 µL for primer (0.8 pmol/µL). If your DNA is not concentrated enough and you need to add more than 8 µL of DNA template, then you can compensate for the additional volume by using a more concentrated solution of primer.

For example, if your concentration of primers is increased from 0.8 pmol/µL to 3.2 pmol/µL, then the volume of primers can be reduced from 4 µL to 1 µL. Because less volume is used for the primers, more volume can then be added for the template. In this example, the volume of DNA template could be increased from 8 µL to 11 µL.

Performing Cycle Sequencing 3

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Topic	See Page
Introduction	3-1
Cycle Sequencing Single- and Double-Stranded DNA	3-2
Cycle Sequencing BAC DNA	3-5

Introduction

Overview The cycle sequencing protocols used for the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS, are the same as those used for the ABI PRISM® BigDye™ Terminator original and v2.0 Ready Reaction Cycle Sequencing Kits. They have been optimized for Applied Biosystems thermal cyclers.

Cycle Sequencing Single- and Double-Stranded DNA

Overview

This section describes how to prepare reactions and perform cycle sequencing on a variety of templates, including M13, plasmids, and PCR products.

Preparing the Reactions for 96-Well Reaction Plates or Microcentrifuge Tubes

The type of tube required depends on the thermal cycler that you are using. Refer to “Materials for Cycle Sequencing” on page 1-12.

To prepare the reaction mixtures:

Step	Action	
1	For each reaction add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	8.0 µL
	Template	See the table in “Template Quantity” on page 2-6.
	Primer	3.2 pmol
	Deionized water	*q.s.*
	Total Volume	20 µL
2	Mix well and spin briefly.	
3	**If using the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:**	
	Overlay reaction mixture with 40 µL of light mineral oil.	

Preparing the Reactions for 384-Well Reaction Plates

The type of tube required depends on the thermal cycler that you are using. Refer to “Materials for Cycle Sequencing” on page 1-12.

Note The wells in a 384-well reaction plate have a volume capacity of 35 µL. Therefore, we recommend doing a 10 µL reaction. This allows the post-reaction cleanup step, which requires 26 µL of volume, to be performed in the same well.

To prepare the reaction mixtures:

Step	Action	
1	For each reaction add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	4.0 µL
	Template	See the table in “Template Quantity” on page 2-6.
	Primer	3.2 pmol
	Deionized water	*q.s.*
	Total Volume	10 µL
2	Mix well and spin briefly.	
3	Use on a GeneAmp® PCR System 9700 Dual 384-Well Sample Block Module.	

Cycle Sequencing on the System 9700, 9600, or 2400

To sequence single- and double-stranded DNA on the GeneAmp® PCR System 9700 (in 9600 emulation mode), 9600, or 2400:

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 20 µL.
2	Repeat the following for 25 cycles: • Rapid thermal ramp[a] to 96 °C • 96 °C for 10 seconds. • Rapid thermal ramp to 50 °C • 50 °C for 5 seconds. • Rapid thermal ramp to 60 °C • 60 °C for 4 minutes
3	Rapid thermal ramp to 4 °C and hold until ready to purify.
4	Spin down the contents of the tubes in a microcentrifuge.
5	Proceed to Chapter 4, “Purifying Extension Products.”

a. Rapid thermal ramp is 1 °C/second.

Cycle Sequencing on the TC1 or 480

To sequence single- and double-stranded DNA on the DNA Thermal Cycler (TC1) or DNA Thermal Cycler 480:

Step	Action
1	Place the tubes in a thermal cycler.
2	Repeat the following for 25 cycles: • Rapid thermal ramp[a] to 96 °C • 96 °C for 30 seconds. • Rapid thermal ramp to 50 °C • 50 °C for 15 seconds. • Rapid thermal ramp to 60 °C • 60 °C for 4 minutes
3	Rapid thermal ramp to 4 °C and hold until ready to purify.
4	Spin down the contents of the tubes in a microcentrifuge.
5	Proceed to Chapter 4, “Purifying Extension Products.”

a. Rapid thermal ramp is 1 °C/second.

Cycle Sequencing BAC DNA

Overview

This section describes how to prepare reactions and perform cycle sequencing on BAC DNA.

BAC DNA Cleanup

Sequencing reactions for BAC DNA require double the quantity of terminator Ready Reaction Mix when compared with sequencing reactions for plasmid DNA. For this reason, we recommend cleaning up the BAC DNA by using spin column purification followed by ethanol precipitation. This will help ensure that excess unincorporated dye terminators are removed.

Thermal Cyclers

Only the GeneAmp PCR Systems 9700 (in 9600 emulation mode) or 9600 thermal cyclers can be used with this protocol. You must re-optimize this protocol for use on other thermal cyclers.

Preparing Sequencing Reactions

The type of tube required depends on the thermal cycler that you are using. Refer to “Materials for Cycle Sequencing” on page 1-12.

To prepare the sequencing reaction:

Step	Action	
1	For each reaction, add the following reagents to a separate tube:	
	Reagent	**Quantity**
	Terminator Ready Reaction Mix	16 µL
	DNA Template	0.5–1.0 µg
	Primer	5–10 pmol
	Deionized water	*q.s.*
	Total Volume	40 µL
2	Mix well and spin briefly.	

Performing Cycle Sequencing

To perform cycle sequencing on BAC DNA:

Step	Action
1	Place the tubes in a thermal cycler and set the volume to 40 µL.
2	Heat the tubes at 95 °C for 5 minutes.
3	Repeat the following for 50 cycles:[a] • Rapid thermal ramp[b] to 95 °C • 95 °C for 30 seconds. • Rapid thermal ramp to 50–55 °C (depending on template) • 50–55 °C for 10 seconds. • Rapid thermal ramp to 60 °C • 60 °C for 4 minutes.
4	Rapid thermal ramp to 4 °C and hold until ready to purify.
5	Spin down the contents of the tubes in a microcentrifuge.
6	Proceed to Chapter 4, “Purifying Extension Products.”

a. Some laboratories have found that increasing the number of cycles gives better results.

b. Rapid thermal ramp is 1 °C/sec.

Purifying Extension Products

4

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Topic	See Page
Choosing a Method of Purification	4-2
Ethanol/Sodium Acetate Precipitation in 96-Well Reaction Plates	4-3
Ethanol/Sodium Acetate Precipitation in Microcentrifuge Tubes	4-5
Ethanol Precipitation in 96-Well Reaction Plates	4-8
Ethanol Precipitation in Microcentrifuge Tubes	4-10
Two Precipitation Methods for 384-Well Reaction Plates	4-12
Plate and Spin Column Purification	4-16

Choosing a Method of Purification

Purpose

Unincorporated dye terminators must be completely removed before the samples can be analyzed by electrophoresis. Excess dye terminators in sequencing reactions obscure data in the early part of the sequence and can interfere with basecalling.

Purification Methods

Because of the new dyes that are used in the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit, current alcohol precipitation methods may not remove unincorporated dyes efficiently. The methods recommended below have produced clean sequencing data. Other precipitation methods are currently being investigated.

Purification Method	See page
Ethanol/Sodium Acetate Precipitation in 96-Well Reaction Plates	4-3
Ethanol/Sodium Acetate Precipitation in Microcentrifuge Tubes	4-5
Ethanol Precipitation in 96-Well Reaction Plates	4-8
Ethanol Precipitation in Microcentrifuge Tubes	4-10
Two Precipitation Methods for 384-Well Reaction Plates	4-12
Plate and Spin Column Purification	4-16

Plate and Spin Column vs. Precipitation

Use the method that works best for your particular application.

- Precipitation methods are cheaper and faster. However, they may remove less of the unincorporated dye-labeled terminators, which can obscure data at the beginning of the sequence.
- The plate and spin column procedures remove more terminators, but are more costly and may take additional time to perform.

Ethanol/Sodium Acetate Precipitation in 96-Well Reaction Plates

Recommended Protocol

With the BigDye terminators v3.0, the ethanol/sodium acetate precipitation method for 96-well reaction plates produces consistent signal, while minimizing unincorporated dyes. A final 70% ethanol wash is required.

Note While this method produces the cleanest signal, it may cause loss of small molecular weight fragments.

Precipitating in 96-Well Reaction Plates

Note A second protocol for precipitating in 96-well reaction plates is located on page 4-8, "Ethanol Precipitation in 96-Well Reaction Plates."

IMPORTANT Use non-denatured 95% ethanol rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

To precipitate in 96-well MicroAmp® reaction plates:

Step	Action
1	Remove the 96-well reaction plate from the thermal cycler. Remove the caps from each tube.
2	Prepare the ethanol/sodium acetate solution by combining the following for each sample: **Note** Make enough to precipitate all samples in your experiment. • 3.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 • 62.5 µL of non-denatured 95% ethanol (EtOH) • 14.5 µL of deionized water The final volume should be 80 µL for each sample. **⚠ WARNING CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Add 80 µL of this ethanol/sodium acetate solution to 20 µL of reaction mixture.
4	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 431 or 439 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.

To precipitate in 96-well MicroAmp® reaction plates: *(continued)*

Step	Action
5	Invert the plate a few times to mix.
6	Leave the plate at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
7	Place the plate in a table-top centrifuge with a tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: • 1400–2000 × *g*: 45 minutes • 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp plate can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
8	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.
9	Place the inverted plate with the paper towel into the table-top centrifuge and spin at 50 × *g* for 1 minute.
10	Add 150 µL of 70% ethanol to each pellet.
11	Cap or seal the tubes, then invert the plate a few times to mix.
12	Spin the plate for 10 minutes at maximum speed (see step 7 above).
13	Repeat steps 8 and 9.
14	Remove the plate and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Ethanol/Sodium Acetate Precipitation in Microcentrifuge Tubes

Recommended Protocol

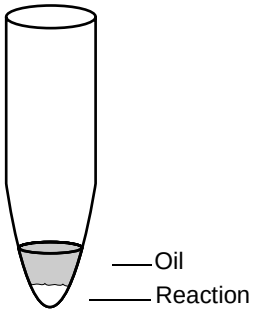
With the BigDye terminators v3.0, the ethanol/sodium acetate precipitation method in microcentrifuge tubes produces consistent signal, while minimizing unincorporated dyes. A final 70% ethanol wash is required.

Note While this method produces the cleanest signal, it may cause loss of small molecular weight fragments.

Precipitating in Microcentrifuge Tubes

IMPORTANT Use non-denatured 95% ethanol rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

To precipitate in microcentrifuge tubes:

Step	Action
1	**IMPORTANT** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as described below. **To remove reactions run on the TC1 or DNA Thermal Cycler 480:** Place the pipette tip into the bottom of the reaction and carefully remove the reaction from the oil. Transfer as little oil as possible. 

To precipitate in microcentrifuge tubes: *(continued)*

Step	Action
2	Prepare the ethanol/sodium acetate solution by combining the following for each sample: • 3.0 µL of 3 M sodium acetate (NaOAc), pH 4.6 • 62.5 µL of non-denatured 95% ethanol (EtOH) • 14.5 µL of deionized water The final volume should be 80 µL for each sample. **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Add 80 µL of this ethanol/sodium acetate solution to 20 µL of reaction mixture.
4	Close the tubes and vortex briefly.
5	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.
6	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Carefully aspirate the supernatants with a separate pipette tip for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
8	Add 250 µL of 70% ethanol to the tubes and mix briefly.
9	Place the tubes in the microcentrifuge in the same orientation as step 5 and spin for 5 minutes at maximum speed.
10	Aspirate the supernatants carefully, as in step 6.

To precipitate in microcentrifuge tubes: *(continued)*

Step	Action
11	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. Do not over-dry.

Ethanol Precipitation in 96-Well Reaction Plates

Unincorporated Terminators

With ethanol precipitation, residual terminator peaks may be seen. However, the recovery of small molecular weight fragments will be improved using this precipitation method.

Precipitating in 96-Well Reaction Plates

Note A second protocol for precipitating in 96-well reaction plates is located on page 4-3, "Ethanol/Sodium Acetate Precipitation in 96-Well Reaction Plates."

IMPORTANT Where 95% ethanol is recommended in precipitation protocols, purchase non-denatured ethanol at this concentration rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

To precipitate in 96-well MicroAmp plates:

Step	Action
1	Remove the MicroAmp plate from the thermal cycler. Remove the caps from each tube.
2	Add the following for each sample: • 16 µL of deionized water • 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%. **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Seal the tubes with strip caps or by applying a piece of 3M Scotch Tape 431 or 439 adhesive-backed aluminum foil tape. Press the foil onto the tubes to prevent any leakage.
4	Invert the plate a few times to mix.
5	Leave the plate at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.

To precipitate in 96-well MicroAmp plates: *(continued)*

Step	Action
6	Place the plate in a table-top centrifuge with a tube-tray adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: • 1400–2000 × *g*: 45 minutes • 2000–3000 × *g*: 30 minutes **Note** A MicroAmp tube in a MicroAmp plate can withstand 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.
8	Place the inverted plate with the paper towel into the table-top centrifuge and spin at 50 × *g* for 1 minute.
9	Add 150 µL of 70% ethanol to each pellet.
10	Cap or seal the tubes, then invert the plate a few times to mix.
11	Spin the plate for 10 minutes at maximum speed. See step 6 above.
12	Repeat steps 7 and 8.
13	Remove the plate and discard the paper towel. **Note** Pellets may or may not be visible. Vacuum drying of the samples is not necessary.

Ethanol Precipitation in Microcentrifuge Tubes

Unincorporated Terminators

With ethanol precipitation, residual terminator peaks may be seen. However, the recovery of small molecular weight fragments will be improved using this precipitation method.

Precipitating in Microcentrifuge Tubes

IMPORTANT Where 95% ethanol is recommended in precipitation protocols, purchase non-denatured ethanol at this concentration rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

To precipitate in microcentrifuge tubes:

Step	Action
1	Pipet the entire contents of each extension reaction into a 1.5-mL microcentrifuge tube. **Note** If the TC1 or DNA Thermal Cycler 480 was used for thermal cycling, remove the reactions from the tubes as shown in step 1 on page 4-5.
2	Add the following for each sample: • 16 µL of deionized water • 64 µL of non-denatured 95% ethanol The final ethanol concentration should be 60 ± 3%. **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Close the tubes and vortex briefly.
4	Leave the tubes at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.

To precipitate in microcentrifuge tubes: *(continued)*

Step	Action
5	Place the tubes in a microcentrifuge and mark their orientations. Spin the tubes for 20 minutes at maximum speed. **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the tubes for 2 minutes more immediately before performing the next step.
6	Carefully aspirate the supernatants with a separate pipette tip for each sample and discard. Pellets may or may not be visible. **IMPORTANT** The supernatants must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tubes, the more unincorporated dye terminators will remain in the samples.
7	Add 250 µL of 70% ethanol to the tubes and vortex them briefly.
8	Place the tubes in the microcentrifuge in the same orientation as in step 5 and spin for 10 minutes at maximum speed.
9	Aspirate the supernatants carefully, as in step 6.
10	Dry the samples in a vacuum centrifuge for 10–15 minutes or to dryness. Do not over-dry.

Two Precipitation Methods for 384-Well Reaction Plates

Two Methods

There are two recommended methods for ethanol/sodium acetate precipitation in 384-well reaction plates.

IMPORTANT Use non-denatured 95% ethanol rather than absolute (100%) ethanol. Absolute ethanol absorbs water from the atmosphere, gradually decreasing its concentration. This can lead to inaccurate final concentrations of ethanol, which can affect some protocols.

Precipitating in 384-Well Reaction Plates: Method 1

To precipitate in 384-well reaction plates (method 1):

Step	Action
1	Remove the 384-well reaction plates from the thermal cycler. Remove the seal from each plate.
2	To 10 µL[a] of reaction mixture, add the following: • 25 µL of non-denatured 95% ethanol (EtOH) • 1 µL of 250 mM EDTA The final ethanol concentration should be 66%. The final reaction volume should be 36 µL. **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION** **CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Seal the plates with a piece of 3M Scotch Tape 431 or 439 adhesive-backed aluminum foil tape. Press the foil onto the plates to prevent any leakage.
4	Invert the plate a few times to mix.
5	Leave the plate at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.

To precipitate in 384-well reaction plates (method 1): *(continued)*

Step	Action
6	Place the plate in a table-top centrifuge with a plate adaptor and spin it at the maximum speed, which must be ≥1400 × *g* but <3000 × *g*: • 1400–2000 × *g*: 45 minutes • 2000–3000 × *g*: 30 minutes **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the plate for 2 minutes more immediately before performing the next step.
7	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.
8	Place the inverted plate with the paper towel into the table-top centrifuge and spin at 20 × *g* for 1 minute.
9	Remove the plate and discard the paper towel. Pellets may or may not be visible.
10	**Note** If you have problems with residual terminator peaks, the pellets can be rinsed with 70% ethanol before drying. Samples can be dried by: • Placing in a Speed-Vac for 15 minutes OR • Air drying at room temperature for 1 hour **IMPORTANT** Make sure the samples are protected from light while they are drying.

a. This protocol ensures that you will not exceed the volume capacity of the 384-well reaction plates.

Precipitating in 384-Well Reaction Plates: Method 2

To precipitate in 384-well reaction plates (method 2):

Step	Action
1	Remove the 384-well reaction plates from the thermal cycler. Remove the seal from each plate.
2	To 10 µL[a] of reaction mixture, add 1 µL of 250 mM EDTA and mix. **⚠ CAUTION** **CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Prepare the ethanol/sodium acetate solution by combining the following for each sample: • 1 µL of 3 M sodium acetate (NaOAc), pH 4.6 • 23 µL of non-denatured 95% ethanol (EtOH) • 1 µL of deionized water The final ethanol concentration should be 62%. The final reaction volume should be 36 µL for each sample. **⚠ WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. It may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin. Exposure may cause central nervous system depression and liver damage. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4	Add 25 µL of this ethanol/sodium acetate solution to the reaction mixture.
5	Seal the plates with a piece of 3M Scotch Tape 431 or 439 adhesive-backed aluminum foil tape. Press the foil onto the plates to prevent any leakage.
6	Invert the plate a few times to mix.
7	Leave the plate at room temperature for 15 minutes to precipitate the extension products. **Note** Precipitation times <15 minutes will result in the loss of very short extension products. Precipitation times >24 hours will increase the precipitation of unincorporated dye terminators.

To precipitate in 384-well reaction plates (method 2): *(continued)*

Step	Action
8	Place the plate in a table-top centrifuge with a plate adaptor and spin it at 3000 × *g* for 30 minutes. **IMPORTANT** Proceed to the next step immediately. If this is not possible, then spin the plate for 2 minutes more immediately before performing the next step.
9	Without disturbing the precipitates, remove the adhesive tape and discard the supernatant by inverting the plate onto a paper towel folded to the size of the plate.
10	Place the inverted plate with the paper towel into the table-top centrifuge and spin at 20 × *g* for 1 minute.
11	Remove the plate and discard the paper towel. Pellets may or may not be visible.
12	**Note** If you have problems with residual terminator peaks, the pellets can be rinsed with 70% ethanol before drying. Samples can be dried by: • Placing in a Speed-Vac for 15 minutes OR • Air drying at room temperature for 1 hour **IMPORTANT** Make sure the samples are protected from light while they are drying.

a. This protocol ensures that you will not exceed the volume capacity of the 384-well reaction plates.

Plate and Spin Column Purification

Overview

This section describes the recommended plate and spin columns for purifying extension products.

IMPORTANT Extra caution is required when dispensing samples onto the column bed. Residual dye peaks will result if samples flow through the sides of the column.

Recommended 96-Well Plate Columns

For large-scale procedures, you can use the following commercially available 96-well purification plates:

- 96-Well plate columns, Gel Filtration Kit (Edge Biosystems, P/N 94880)
- Centri-Sep™ 96 plate columns (Princeton Separations, P/N CS-961).

Performing 96-Well Plate Column Purification

For 96-well plate column purification methods, follow the manufacturer's recommended protocols.

Note Methods for 384-well plate column purification are currently being evaluated.

Recommended Spin Columns

We recommend Centri-Sep™ spin columns (Applied Biosystems, P/N 401763 for 32 columns and P/N 401762 for 100 columns).

Optimizing Spin Column Purification

IMPORTANT When using the BigDye terminators v3.0, hydrate the column for 2 hours.

Tips for optimizing spin column purification when using individual columns:

- Do not process more columns than you can handle conveniently at one time.
- Load the sample in the center of the column bed slowly. Make sure that the sample does not touch the sides of the column and that the pipet tip does not touch the gel surface.

  If samples are not properly loaded, peaks from unincorporated dye terminators can result.

- Spin the column at 325–730 × *g* for best results. Use the following formula to calculate the best speed for your centrifuge:

  $g = 11.18 \times r \times (\text{rpm}/1000)^2$

  where:

  g = relative centrifugal force

  r = radius of the rotor in cm

  rpm = revolutions per minute
- Do not spin for more than 2 minutes.
- Perform the entire procedure without interruption to ensure optimal results. Do not allow the column to dry out.

Performing Spin Column Purification

To perform spin column purification:

Step	Action
1	Gently tap the column to cause the gel material to settle to the bottom of the column.
2	Remove the upper end cap and add 0.8 mL of deionized water.
3	Replace the upper end cap and vortex or invert the column a few times to mix the water and gel material.
4	Allow the gel to hydrate at room temperature for at least 2 hours. **Note** Hydrated columns can be stored for a few days at 2–6 °C. Longer storage in water is not recommended. Allow columns stored at 2–6 °C to warm to room temperature before use.
5	Remove any air bubbles by inverting or tapping the column and allowing the gel to settle.
6	Remove the upper end cap first, then remove the bottom cap. Allow the column to drain completely by gravity. **Note** If flow does not begin immediately, apply gentle pressure to the column with a pipette bulb.
7	Insert the column into the wash tube provided.
8	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes to remove the interstitial fluid.
9	Remove the column from the wash tube and insert it into a sample collection tube (*e.g.*, a 1.5-mL microcentrifuge tube).
10	Remove the extension reaction mixture from its tube and load it carefully onto the center of the gel material.

To perform spin column purification: *(continued)*

Step	Action
11	Spin the column in a microcentrifuge at 730 × *g* for 2 minutes. **Note** If using a centrifuge with a fixed-angle rotor, place the column in the same orientation as it was in for the first spin. This is important because the surface of the gel will be at an angle in the column after the first spin.
12	Discard the column. The sample is in the sample collection tube.
13	Dry the sample in a vacuum centrifuge for 10–15 minutes, or until dry. Do not over-dry.

Sample Electrophoresis 5

Chapter Summary

In This Chapter The following topics are covered in this chapter:

Topic	See Page
Before You Begin	5-2
Electrophoresis on the ABI PRISM 3700 DNA Analyzer	5-3
Electrophoresis on the ABI PRISM 3100 Genetic Analyzer	5-4
Electrophoresis on the ABI PRISM 310 Genetic Analyzer	5-5
Electrophoresis on the ABI PRISM 377 DNA Sequencers	5-8
Electrophoresis on the 373 Instrument with BigDye Filter Wheel	5-11

Before You Begin

Important Reminders

- Dye set/primer (mobility) file names for the BigDye™ terminators v3.0 are different than those for the dRhodamine terminators and BigDye terminators original and v2.0.
- If a mobility file for the wrong sequencing chemistry is used, some bases may be miscalled. This is due to different dye labeling for the different chemistries. In addition, there are differences in the mobility shifts between the dRhodamine and BigDye terminator v3.0 chemistries.
- Use the same dye set/primer (mobility) files for BigDye terminators v3.0 and dGTP BigDye terminators v3.0.

Note See “Dye Set/Primer (Mobility) Files” on page 1-7 for information on obtaining the v3.0 dye set/primer (mobility) files.

Electrophoresis on the ABI PRISM 3700 DNA Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 3700 DNA Analyzer requires the following:

Run Modules

Configuration	Run Module
POP-5™ polymer, 50-cm	Seq1_1POP5DefaultModule
	Seq1_2POP5DefaultModule
POP-6™ polymer, 50-cm	Seq1_1POP6DefaultModule
	Seq1_2POP6DefaultModule

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-5 polymer	DT3700POP5{BDv3}v1.mob
POP-6 polymer	DT3700POP6{BDv3}v1.mob

Standards

IMPORTANT Use Dye Set D.

Dye Set	Standards for Spectral Calibration
D	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390309)

Note Refer to the product insert for instructions on using the standards for this instrument.

Performing Sample Electrophoresis

For information on how to perform sample electrophoresis on the 3700 instrument, refer to the following manuals:

- *ABI PRISM 3700 DNA Analyzer Sequencing Chemistry Guide* (P/N 4309125)
- *ABI PRISM 3700 DNA Analyzer User's Manual* (P/N 4306152)

Electrophoresis on the ABI PRISM 3100 Genetic Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 3100 Genetic Analyzer requires the following:

Run Modules

Configuration	Run Module
POP-6™ polymer, 36-cm	RapidSeq36_POP6DefaultModule
POP-6 polymer, 50-cm	StdSeq50_POP6DefaultModule

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-6™ polymer	DT3100POP6{BDv3}v1.mob

Standards

IMPORTANT Use Dye Set Z.

Dye Set	Standards for Spectral Calibration
Z	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard (P/N 4390303)

Note Refer to the product insert for instructions on using the standards for this instrument.

Performing Sample Electrophoresis

For information on how to perform sample electrophoresis on the 3100 instrument, refer to the following manuals:

- *ABI PRISM 3100 Genetic Analyzer Sequencing Chemistry Guide* (P/N 4315831)
- *ABI PRISM 3100 Genetic Analyzer User's Manual* (P/N 4315834)

Electrophoresis on the ABI PRISM 310 Genetic Analyzer

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 310 Genetic Analyzer requires the following:

Filter Set E Run Modules

Configuration	Run Module
POP-4™ polymer, 1-mL syringe, 47-cm, 50-µm i.d. capillary, Ld = 36 cm	P4StdSeq (1 mL) E
POP-4 polymer, Rapid Sequencing, 1-mL syringe, 47-cm, 50-µm i.d. capillary, Ld = 36 cm	P4RapidSeq (1 mL) E
POP-6™ polymer, 1-mL syringe, 61-cm, 50-µm i.d. capillary	Seq POP6 (1 mL) E
POP-6 polymer, Rapid Sequencing, 1-mL syringe, 47-cm, 50-µm i.d. capillary	Seq POP6 Rapid (1 mL) E

Dye Set/Primer (Mobility) Files

Polymer	Dye Set/Primer (Mobility) File
POP-4 polymer	DT310POP4{BDv3}v1.mob
POP-6 polymer	DT310POP6{BDv3}v1.mob

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye terminators v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye™ primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note Refer to the product insert for instructions on using the standards for this instrument.

Resuspending the Samples for Sequencing with POP-6 Polymer

To resuspend the samples for sequencing with POP-6 polymer:

Step	Action
1	Resuspend each sample pellet in 25 µL of template suppression reagent (TSR, supplied with the polymer). **⚠ CAUTION CHEMICAL HAZARD**. **Template suppression reagent** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex and spin the samples.
3	Heat the samples at 95 °C for 2 minutes to denature, then chill on ice.
4	Vortex and spin the samples again. Place on ice until ready to use.
5	Refer to the *ABI PRISM 310 Genetic Analyzer User's Manual* (P/N 903565) for guidelines on loading the samples.

Note Although not recommended on a routine basis, you can keep samples prepared in TSR frozen for several weeks before running on the ABI PRISM 310 Genetic Analyzer with no detectable loss in resolution or base calling.

Resuspending the Samples for Sequencing with POP-4 Poymer

Deionized formamide is the recommended resuspension solution for sequencing with POP-4 polymer on the 310 genetic analyzer.

IMPORTANT Use only the highest grade of deionized formamide, such as Applied Biosystems Hi-Di™ formamide (P/N 4311320). Deionized formamide should be stored at –20 °C in usable aliquots to prevent several freeze-thaw cycles. If the deionized formamide stored at –20 °C does not freeze, discard it and use fresh deionized formamide for sample resuspension.

To resuspend the samples for sequencing with POP-4 polymer:

Step	Action
1	Resuspend each sample pellet in 25 µL of deionized formamide. **IMPORTANT** Securely seal each sample tube after resuspension with deionized formamide to limit the sample's exposure to air. **⚠ WARNING CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2	Vortex and spin the samples.
3	Heat the samples at 95 °C for 2 minutes to denature, then chill on ice.
4	Vortex and spin the samples again. Place on ice until ready to use.
5	Refer to the ABI PRISM 310 *Genetic Analyzer User's Manual* (P/N 903565) for guidelines on loading the samples.

Note Although extended sample storage at room temperature is not recommended on a routine basis, you can keep securely sealed samples prepared in deionized formamide at room temperature for up to 48 hours on the ABI PRISM 310 Genetic Analyzer autosampler with no detectable loss in resolution or base calling. Freezing of samples resuspended in deionized formamide is not advised.

Electrophoresis on the ABI PRISM 377 DNA Sequencers

Requirements

Electrophoresis and data analysis of samples on the ABI PRISM® 377 DNA Sequencers (all models[1]) require the following:

Filter Set E Run Modules

Configuration[a]	Run Module
36-cm wtr, 1200 scans/hr, any comb	Seq Run 36E-1200
36-cm wtr, 2400 scans/hr, any comb	Seq Run 36E-2400
48-cm wtr, 1200 scans/hr, any comb	Seq Run 48E-1200

a. Any plate check and prerun module can be used on the ABI PRISM 377 DNA Sequencers.

Dye Set/Primer (Mobility) Files

Gel Formulation	Dye Set/Primer (Mobility) File
4.5% acrylamide (29:1) or 5% Long Ranger™ gel	DT377{BDv3}v1.mob

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye terminators v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
E	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note Refer to the product insert for instructions on using the standards for this instrument.

1. Includes the ABI PRISM 377, ABI PRISM 377-18, ABI PRISM 377 with XL Upgrade, and the ABI PRISM 377 with 96-Lane Upgrade instruments.

Using the Lane Guide Kit

If you are using the BigDye chemistries v3.0 on the 377 instrument in conjunction with the ABI PRISM® Lane Guide™ Lane Identification Kit, refer to that kit's protocol (P/N 4313804) for instructions on resuspending and loading samples.

Using Long-Read Gel and Buffer Formulations

For longer sequencing read lengths follow the gel and buffer formulations described in the user bulletin entitled *Achieving Longer High Accuracy Reads on the 377 Sequencer* (P/N 4315153).

Resuspending and Loading the Samples

Note You can use any plate check and prerun modules.

To resuspend and load the samples:

Step	Action		
1	Prepare a loading buffer by combining the following in a 5:1 ratio (5 parts deionized formamide to 1 part EDTA with blue dextran): • Deionized formamide • 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL) **⚠ WARNING** **CHEMICAL HAZARD**. **Formamide** is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **⚠ CAUTION** **CHEMICAL HAZARD. EDTA** may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.		
2	Resuspend each sample pellet in loading buffer as follows:		
	Template	**Volume (µL): 18- or 36-well**	**Volume (µL): 48-, 64-, or 96-well**
	PCR product, plasmid, M13	6–8	4–6
	BAC, large DNA	2	1.5
3	Vortex and spin the samples.		
4	Heat the samples at 95 °C for 2 minutes to denature. Place on ice until ready to load.		

To resuspend and load the samples: *(continued)*

Step	Action		
5	Load each sample into a separate lane of the gel as follows:		
	Template	**Volume (µL): 18- or 36-well**	**Volume (µL): 48-, 64-, or 96-well**
	PCR product, plasmid, M13	0.75–2.0	0.5–1.5
	BAC, large DNA	2	48-well: 2.0 64-well: 1.5 96-well: 1.0–1.5

Note If a weak signal is obtained on the ABI PRISM 377 DNA Sequencer with XL Upgrade, rerun the samples using a CCD gain of 4. Refer to the *ABI PRISM 377 DNA Sequencer XL Upgrade User's Manual* (P/N 904412) for more information.

Electrophoresis on the 373 Instrument with BigDye Filter Wheel

Requirements

General guidelines are provided below for running the ABI PRISM® 373 DNA Sequencers with the ABI PRISM® BigDye™ Filter Wheel[2] installed. For more detailed instructions, please refer the user's manual for your 373 instrument or to the user bulletin entitled *Using the ABI PRISM 373 BigDye Filter Wheel* (P/N 4304367).

Gel

For 48-cm well-to-read (wtr), we recommended 5% Long Ranger™ gel.

New Dye Set/Primer (Mobility) Files

Gel Formulation	Dye Set/Primer (Mobility) File
5% Long Ranger gel, 48-cm wtr[a]	DT373{BDv3}v1.mob

a. If you are running other wtr lengths, these are being tested. Please call Technical Support.

Matrix Standards

IMPORTANT The instrument (matrix) file for the BigDye terminators v3.0 cannot be used for the BigDye terminators (original), BigDye terminators v2.0, dRhodamine terminators, or BigDye primers (original).

Dye/Filter Set	Standards for Instrument (Matrix) File Generation
A (For use with the BigDye Filter Wheel)	ABI PRISM® BigDye™ Matrix Standards v3.0 (P/N 4390421)

Note For instructions on using the matrix standards (P/N 4390421) for the 373 instruments, contact Technical Support.

2. Includes the ABI PRISM 373 and ABI PRISM 373 with XL Upgrade instruments.

Resuspending and Loading the Samples

To resuspend and load the samples:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Prepare a loading buffer by combining the following in a 5:1 ratio (5 parts deionized formamide to 1 part EDTA with blue dextran):
• Deionized formamide
• 25 mM EDTA (pH 8.0) with blue dextran (50 mg/mL)
⚠ WARNING CHEMICAL HAZARD. Formamide is harmful if absorbed through the skin and may cause irritation to the eyes, skin, and respiratory tract. It may cause damage to the central nervous system and the male and female reproductive systems, and is a possible birth defect hazard. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
⚠ CAUTION CHEMICAL HAZARD. EDTA may cause eye, skin, and respiratory tract irritation. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</td></tr>
<tr><td>2</td><td>Resuspend each sample pellet in loading buffer as follows:
<table>
<tr><th rowspan="2">Template</th><th colspan="4">Volume (µL)</th></tr>
<tr><th>18 or 24 well</th><th>32 or 36 well</th><th>48-well</th><th>64-well</th></tr>
<tr><td>PCR product, plasmid, M13</td><td>3–4</td><td>3–4</td><td>2–4</td><td>2–4</td></tr>
<tr><td>BAC, large DNA</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
</table></td></tr>
<tr><td>3</td><td>Vortex and spin the samples.</td></tr>
<tr><td>4</td><td>Heat the samples at 95 °C for 2 minutes to denature. Place on ice until ready to load.</td></tr>
<tr><td>5</td><td>Load each sample into a separate lane of the gel as follows:
<table>
<tr><th rowspan="2">Template</th><th colspan="4">Volume (µL)</th></tr>
<tr><th>18 or 24 well</th><th>32 or 36 well</th><th>48-well</th><th>64-well</th></tr>
<tr><td>PCR product, plasmid, M13</td><td>3–4</td><td>3–4</td><td>2–2.5</td><td>2</td></tr>
<tr><td>BAC, large DNA</td><td>3</td><td>3</td><td>2</td><td>2</td></tr>
</table></td></tr>
</table>

Selecting Sequencing Primers

A

Selecting Sequencing Primers

Overview

The choice of sequencing primer sequence, method of primer synthesis, and approach to primer purification can have a significant effect on the quality of the sequencing data obtained in dye terminator cycle sequencing reactions with this kit.

These decisions are particularly important when sequencing is done on real-time detection systems where signal strength is critical. Some of the recommendations given here are based on information that is general knowledge, while others are based on practical experience gained by Applied Biosystems scientists.

Recommendations

The following recommendations are provided to help optimize primer selection:

- Primers should be at least 18 bases long to ensure good hybridization.
- Avoid runs of an identical nucleotide. This is especially true for guanine, where runs of four or more Gs should be avoided.
- Keep the G-C content in the range 30–80%.
- For cycle sequencing, primers with melting temperatures (T_m) above 45 °C produce better results than primers with lower T_m.
- For primers with a G-C content less than 50%, it may be necessary to extend the primer sequence beyond 18 bases to keep the T_m>45 °C.
- Use of primers longer than 18 bases also minimizes the chance of having a secondary hybridization site on the target DNA.
- Avoid primers that have secondary structure or that can hybridize to form dimers.

- Several computer programs for primer selection are available. They can be useful in identifying potential secondary structure problems and determining if a secondary hybridization site exists on the target DNA.

Control DNA Sequence

B

Control Sequence

Partial Sequence of pGEM-3Zf(+)

The pGEM®-3Zf(+) sequence below is the sequence of the –21 M13 forward primer, followed by the ensuing 1000 bases.

TGTAAAACGACGGCCAGT (–21 M13 primer)

GAATTGTAAT	ACGACTCACT	ATAGGGCGAA	TTCGAGCTCG	40
GTACCCGGGG	ATCCTCTAGA	GTCGACCTGC	AGGCATGCAA	80
GCTTGAGTAT	TCTATAGTGT	CACCTAAATA	GCTTGGCGTA	120
ATCATGGTCA	TAGCTGTTTC	CTGTGTGAAA	TTGTTATCCG	160
CTCACAATTC	CACACAACAT	ACGAGCCGGA	AGCATAAAGT	200
GTAAAGCCTG	GGGTGCCTAA	TGAGTGAGCT	AACTCACATT	240
AATTGCGTTG	CGCTCACTGC	CCGCTTTCCA	GTCGGGAAAC	280
CTGTCGTGCC	AGCTGCATTA	ATGAATCGGC	CAACGCGCGG	320
GGAGAGGCGG	TTTGCGTATT	GGGCGCTCTT	CCGCTTCCTC	360
GCTCACTGAC	TCGCTGCGCT	CGGTCGTTCG	GCTGCGGCGA	400
GCGGTATCAG	CTCACTCAAA	GGCGGTAATA	CGGTTATCCA	440
CAGAATCAGG	GGATAACGCA	GGAAAGAACA	TGTGAGCAAA	480
AGGCCAGCAA	AAGGCCAGGA	ACCGTAAAAA	GGCCGCGTTG	520
CTGGCGTTTT	TCCATAGGCT	CCGCCCCCCT	GACGAGCATC	560
ACAAAAATCG	ACGCTCAAGT	CAGAGGTGGC	GAAACCCGAC	600
AGGACTATAA	AGATACCAGG	CGTTTCCCCC	TGGAAGCTCC	640
CTCGTGCGCT	CTCCTGTTCC	GACCCTGCCG	CTTACCGGAT	680
ACCTGTCCGC	CTTTCTCCCT	TCGGGAAGCG	TGGCGCTTTC	720
TCATAGCTCA	CGCTGTAGGT	ATCTCAGTTC	GGTGTAGGTC	760
GTTCGCTCCA	AGCTGGGCTG	TGTGCACGAA	CCCCCCGTTC	800
AGCCCGACCG	CTGCGCCTTA	TCCGGTAACT	ATCGTCTTGA	840
GTCCAACCCG	GTAAGACACG	ACTTATCGCC	ACTGGCAGCA	880
GCCACTGGTA	ACAGGATTAG	CAGAGCGAGG	TATGTAGGCG	920
GTGCTACAGA	GTTCTTGAAG	TGGTGGCCTA	ACTACGGCTA	960
CACTAGAAGG	ACAGTATTTG	GTATCTGCGC	TCTGCTGAAG	1000

Technical Support

C

Technical Support

Contacting Technical Support

You can contact Applied Biosystems for technical support by telephone or fax, by e-mail, or through the Internet. You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems Web site (please see the section “To Obtain Documents on Demand” following the telephone information below).

To Contact Technical Support by E-Mail

Contact technical support by e-mail for help in the following product areas:

Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide and DNA Synthesis	corelab@appliedbiosystems.com
Biochromatography, PerSeptive DNA, PNA and Peptide Synthesis systems, CytoFluor®, FMAT™, Voyager™, and Mariner™ Mass Spectrometers	tsupport@appliedbiosystems.com
Applied Biosystems/MDS Sciex	api3-support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

Hours for Telephone Technical Support

In the United States and Canada, technical support is available at the following times:

Product	Hours
Chemiluminescence	8:30 a.m. to 5:30 p.m. Eastern Time
Framingham support	8:00 a.m. to 6:00 p.m. Eastern Time
All Other Products	5:30 a.m. to 5:00 p.m. Pacific Time

To Contact Technical Support by Telephone or Fax

In North America

To contact Applied Biosystems Technical Support, use the telephone or fax numbers given below. (To open a service call for other support needs, or in case of an emergency, dial **1-800-831-6844** and press **1**.)

Product or Product Area	Telephone Dial...	Fax Dial...
ABI PRISM® 3700 DNA Analyzer	**1-800-831-6844,** then press **8**	**1-650-638-5981**
DNA Synthesis	**1-800-831-6844**, then press **21**	**1-650-638-5981**
Fluorescent DNA Sequencing	**1-800-831-6844**, then press **22**	**1-650-638-5981**
Fluorescent Fragment Analysis (includes GeneScan® applications)	**1-800-831-6844**, then press **23**	**1-650-638-5981**
Integrated Thermal Cyclers (ABI PRISM®877 and Catalyst 800 instruments)	**1-800-831-6844**, then press **24**	**1-650-638-5981**
ABI PRISM® 3100 Genetic Analyzer	**1-800-831-6844**, then press **26**	**1-650-638-5981**
BioInformatics (includes BioLIMS®, BioMerge®, and SQL GT™ applications)	**1-800-831-6844**, then press **25**	**1-505-982-7690**
Peptide Synthesis (433 and 43X Systems)	**1-800-831-6844**, then press **31**	**1-650-638-5981**
Protein Sequencing (Procise® Protein Sequencing Systems)	**1-800-831-6844**, then press **32**	**1-650-638-5981**

Product or Product Area	Telephone Dial...	Fax Dial...
PCR and Sequence Detection	**1-800-762-4001**, then press **1** for PCR, **2** for the 7700 or 5700, **6** for the 6700 or dial **1-800-831-6844**, then press **5**	**1-240-453-4613**
Voyager™ MALDI-TOF Biospectrometry™ and Mariner™ ESI-TOF Mass Spectrometry Workstations	**1-800-899-5858**, then press **13**	**1-508-383-7855**
Biochromatography (BioCAD® Workstations and POROS® Perfusion Chromatography Products)	**1-800-899-5858**, then press **14**	**1-508-383-7855**
Expedite™ Nucleic acid Synthesis Systems	**1-800-899-5858**, then press **15**	**1-508-383-7855**
Peptide Synthesis (Pioneer™ and 9050 Plus Peptide Synthesizers)	**1-800-899-5858**, then press **15**	**1-508-383-7855**
PNA Custom and Synthesis	**1-800-899-5858,** then press **15**	**1-508-383-7855**
FMAT™ 8100 HTS System and Cytofluor® 4000 Fluorescence Plate Reader	**1-800-899-5858**, then press **16**	**1-508-383-7855**
Chemiluminescence (Tropix)	**1-800-542-2369** (U.S. only), or **1-781-271-0045**	**1-781-275-8581**
Applied Biosystems/MDS Sciex	**1-800-952-4716**	**1-650-638-6223**

Outside North America

Region	Telephone Dial...	Fax Dial...
Africa and the Middle East		
Africa (English Speaking) and West Asia (Fairlands, South Africa)	**27 11 478 0411**	**27 11 478 0349**
South Africa (Johannesburg)	**27 11 478 0411**	**27 11 478 0349**
Middle Eastern Countries and North Africa (Monza, Italia)	**39 (0)39 8389 481**	**39 (0)39 8389 493**

Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 758 8268**	**60 3 754 9043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 712 5555**	**32 (0)2 712 5516**
Czech Republic and Slovakia (Praha)	**420 2 61 222 164**	**420 2 61 222 168**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0) 6150 101 0**	**49 (0) 6150 101 101**
Hungary (Budapest)	**36 (0)1 270 8398**	**36 (0)1 270 8288**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**

Region	Telephone Dial...	Fax Dial...
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 (22) 866 40 10**	**48 (22) 866 40 20**
Portugal (Lisboa)	**351 (0)22 605 33 14**	**351 (0)22 605 33 15**
Russia (Moskva)	**7 095 935 8888**	**7 095 564 8787**
South East Europe (Zagreb, Croatia)	**385 1 34 91 927**	**385 1 34 91 840**
Spain (Tres Cantos)	**34 (0)91 806 1210**	**34 (0)91 806 1206**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 331400**	**31 (0)180 331409**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**
All other countries not listed (Warrington, UK)	**44 (0)1925 282481**	**44 (0)1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6100**	**81 3 5566 6501**
Latin America		
Del.A. Obregon, Mexico	**305-670-4350**	**305-670-4349**

To Reach Technical Support Through the Internet

We strongly encourage you to visit our Web site for answers to frequently asked questions and for more information about our products. You can also order technical documents or an index of available documents and have them faxed or e-mailed to you through our site. The Applied Biosystems Web site address is

http://www.appliedbiosystems.com/techsupp

To submit technical questions from North America or Europe:

Step	Action
1	Access the Applied Biosystems Technical Support Web site.
2	Under the **Troubleshooting** heading, click **Support Request Forms**, then select the relevant support region for the product area of interest.
3	Enter the requested information and your question in the displayed form, then click **Ask Us RIGHT NOW** (blue button with yellow text).
4	Enter the required information in the next form (if you have not already done so), then click **Ask Us RIGHT NOW**. You will receive an e-mail reply to your question from one of our technical experts within 24 to 48 hours.

To Obtain Documents on Demand

Free, 24-hour access to Applied Biosystems technical documents, including MSDSs, is available by fax or e-mail or by download from our Web site.

To order documents...	Then...
by index number	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Click the **Index** link for the document type you want, then find the document you want and record the index number. c. Use the index number when requesting documents following the procedures below.
by phone for fax delivery	a. From the U.S. or Canada, call **1-800-487-6809,** or from outside the U.S. and Canada, call **1-858-712-0317**. b. Follow the voice instructions to order the documents you want. **Note** There is a limit of five documents per request.
through the Internet for fax or e-mail delivery	a. Access the Applied Biosystems Technical Support Web site at **http://www.appliedbiosystems.com/techsupp** b. Under **Resource Libraries**, click the type of document you want. c. Enter or select the requested information in the displayed form, then click **Search**. d. In the displayed search results, select a check box for the method of delivery for each document that matches your criteria, then click **Deliver Selected Documents Now** (or click the PDF icon for the document to download it immediately). e. Fill in the information form (if you have not previously done so), then click **Deliver Selected Documents Now** to submit your order. **Note** There is a limit of five documents per request for fax deliver but no limit on the number of documents you can order for e-mail delivery.

ABI PRISM® DNA Sequencing Kits and Related Products

To order ABI PRISM DNA sequencing kits, please contact Applied Biosystems at one of the regional sales offices listed on the back of this protocol. All reagents are quality-controlled in stable formulations. All the kits listed below include protocols. Protocols can also be ordered separately.

dRhodamine Terminator Cycle Sequencing Kits with AmpliTaq® DNA Polymerase, FS

P/N	Kit	Reactions
403044	Ready Reaction	100
403045	Ready Reaction	1000
4303143	Ready Reaction	5000
403041	Protocol	–

The ABI PRISM® BigDye™ Primer v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

P/N	Primer	Reactions
4390161	-21 M13	100
4390163	M13 reverse	100
4390157	-21 M13	5000
4390159	M13 reverse	5000

The ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

P/N	Kit	Reactions
4390236	Ready Reaction	24
4390242	Ready Reaction	100
4390244	Ready Reaction	1000
4390246	Ready Reaction	5000
4390253	Ready Reaction	25000

The ABI PRISM® dGTP BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS

P/N	Kit	Reactions
4390229	Ready Reaction	100

ABI PRISM® Lane Guide™ Lane Identification Kits for use on the 377 Sequencer

P/N	Kit	Reactions
4313682	Lane Guide	200
4313677	Lane Guide	1000
4313679	Lane Guide	5000
4313804	Protocol	–

ABI PRISM Matrix Standards

P/N	Kit	Instrument
4390309	ABI PRISM® 3700 BigDye™ Terminator v3.0 Sequencing Standard	3700
4390303	ABI PRISM® BigDye™ Terminator v3.0 Sequencing Standard	3100/377/ 373/310
4390421	ABI PRISM® BigDye™ Matrix Standards v3.0	377/373/310

Applied Biosystems

Regional Sales Offices

THE AMERICAS

United States
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
Tel: (650) 570-6667
(800) 345-5224
Fax: (650) 572-2743

Canada (Mississauga, Ontario)
Tel: (905) 821-8183
(800) 668-6913
Fax: (905) 821-8246

Latin America (Del.A. Obregon, Mexico)
Tel: (305) 670-4350
Fax: (305) 670-4349

EUROPE, AFRICA

Austria (Wien)
Tel: 01 602 3101
Fax: 01 602 5174

Benelux (Nieuwerkerk a/d IJssel, Netherlands)
Tel: 31 (0)180-331400
Fax: 31 (0)180-331409

Chekia Rep. (Praha)
Tel: 2-61 22 21 64
Fax: 2-61 22 21 68

Denmark (Allerød)
Tel: 48 100 400
Fax: 48 100 401

Finland (Espoo)
Tel: 09 751 72 700
Fax: 09 751 72 701

France (Paris)
Tel: 33-1 69 59 85 85
Fax: 33-1 69 59 85 00

Germany (Weiterstadt)
Tel: (0) 6150/101-0
Fax: (0) 6150/101-101

Hungary (Budapest)
Tel: 36-1-258-8410
Fax: 36-1-256-9802

Italy (Milano)
Tel: (039) 23831
Fax: (039) 2383492

Norway (Oslo)
Tel: (0) 22 02 1500
Fax: (0) 22 02 1501

Poland (Warszawa)
Tel: (48 22) 866 4010
Fax: (48 22) 866 4020

Portugal (Lisboa)
Tel: (351-1) 386 0997
Fax: (351-1) 386 1000

Russia (Moskva)
Tel: 095 935 8888
Fax: 095 564 8787

South Africa (Johannesburg)
Tel: 27 11 478 0411
Fax: 27 11 478 0349

Spain (Madrid)
Tel: (91) 806 1200
Fax: (91) 804 0414

Sweden (Stockholm)
Tel: (0)8 619 4400
Fax: (0)8 619 4401

Switzerland (Rotkreuz)
Tel: 041-799 7708
Fax: 041-790 0676

United Kingdom (Warrington, Cheshire)
Tel: (01925) 825650
Fax: (01925) 282502

All Other European Countries, Middle East, West Asia, Africa Except South Africa

Langen, Germany
Tel: 49 6103 708 301
Fax: 49 6103 708 310

EASTERN ASIA, CHINA, OCEANIA, PACIFIC RIM

Australia (Scoresby, Victoria)
Tel: (03) 9212-8585
Fax: (03) 9212-8502

China (Beijing)
Tel: 86 10 6238 1156
Fax: 86 10 6238 1162

Hong Kong
Tel: 852 2756 6928
Fax: 852 2756 6968

Japan (Chiba)
Tel: (0473) 80-8500
Fax: (0473) 80-8505

Korea (Seoul)
Tel: 822 592 7238
Fax: 822 532 4908

Malaysia (Kuala Lumpur)
Tel: 60 3 758 1118
Fax: 60 3 754 9043

Singapore
Tel: 65 896 2118
Fax: 65 896 2147

Taiwan (Taipei Hsisn)
Tel: 886 22 698 3505
Fax: 886 22 698 3405

Thailand (Bangkok)
Tel: 662 719 6406
Fax: 662 319 9788

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